Journal of Medical Virology 49:274–278 (1996)

HLA DRB1 and DQB1 Alleles and Haplotypes Influencing the Progression of Hepatitis C

Tatsuya Aikawa, Maki Kojima, Hiroshi Onishi, Ryoji Tamura, Satoko Fukuda, Takashi Suzuki, Fumio Tsuda, Hiroaki Okamoto, Yuzo Miyakawa, and Makoto Mayumi

Aikawa Internal Hospital (T.A., M.K.); Technical Research Laboratories, Sumitomo Metal Industries, Ltd., Ibaraki-Ken (H.On., R.T.); Japanese Red Cross Tochigi Blood Center, Tochigi-Ken (S.F., T.S.); Department of Medical Sciences, Toshiba General Hospital, Tokyo (F.T.); Immunology Division, Jichi Medical School, Tochigi-Ken, (H.Ok., M.M.); and Miyakawa Memorial Research Foundation, Tokyo (Y.M.), Japan

Some HLA class II alleles and haplotypes were examined by restriction fragment length polymorphism of corresponding DNA fragments amplified by the polymerase chain reaction in 117 patients with chronic hepatitis C in Japan. The prevalence rates were compared between patients and 1216 controls and in 67 patients with liver cirrhosis, of whom 20 had hepatocellular carcinoma and 50 patients with chronic hepatitis who did not have cirrhosis or hepatocellular carcinoma. Notably, DRB1*0405 (49% [95% confidence range 38–60%] vs. 26% [16–40%]; $P < 0.05$, relative risk [rr] = 2.8) and DQB1*0401 (43% [33–54%] vs. 22% [13–34%]; $P < 0.05$, rr = 2.1) were detected more frequently in patients with cirrhosis than in those without cirrhosis. By contrast, DRB1*0901 (11% [6–19%] vs. 28% [18–40%]; $P < 0.05$; rr = 0.3) and DQB1*0303 (11% [6–19%] vs. 36% [25–49%]; $P < 0.01$; rr = 0.2) were detected less frequently in patients with cirrhosis than those without cirrhosis. Accordingly, the DRB1*0405-DQB1*0401 haplotype was more common (43% [33–54%] vs. 22% [13–34%]; $P < 0.05$; rr = 2.7), while the DRB1*0901-DQB1*0303 haplotype was less common (9% [4–17%] vs. 28% [18–40%]; $P < 0.05$; rr = 0.3) in patients with cirrhosis than in those without cirrhosis. These results suggest that there would be HLA class II alleles and haplotypes which may be associated with an accelerated or slower progression of chronic hepatitis C towards cirrhosis and eventually to hepatocellular carcinoma.

KEY WORDS: hepatitis C, hepatitis C viruses, chronic hepatitis, liver cirrhosis, hepatocellular carcinoma, HLA antigens, histocompatibility antigens class II

INTRODUCTION

There is a high rate of posttransfusion hepatitis C in recipients of blood units contaminated with hepatitis C virus (HCV) [Van der Poel et al., 1990]. Subsequently, HCV persists in more than two-thirds of infected individuals [Alter et al., 1989; Dienstag, 1983]. The persistence of HCV infection, however, does not always induce clinical disease in the host. The natural history of HCV infection is as yet not clear except that it involves a mild disease but it can progress to severe hepatic disease leading to liver cirrhosis and hepatocellular carcinoma in the long run.

Evidence is accumulating to indicate that, like hepatitis B, the pathogenesis of HCV-associated liver disease is mediated by cellular immune responses of hosts [Jung et al., 1994; Rice and Walker, 1995]. HLA class I-restricted, CD8+ T-cell responses have been demonstrated by lymphocytes in peripheral blood and liver against epitopes in the core and envelope proteins as well as non-structural proteins of HCV [Battegay et al., 1995; Cerny et al., 1995; Kita et al., 1993; Koziel et al., 1993; Koziel et al., 1992; Shirai et al., 1995]. Likewise, HLA class II-restricted CD4+ T-cell proliferation has been reported in response to various recombinant HCV proteins [Botarelli et al., 1993; Minutello et al., 1993; Schupper et al., 1993]. Inasmuch as cellular immune responses are implicated in the induction and maintenance of HCV-associated liver disease as well as the prevention of HCV infection the perpetuation of liver disease in persons infected with HCV may correlate with some HLA types.

Consequently, a number of HLA class II genes were typed and haplotypes examined by restriction fragment length polymorphism of DNA fragments, amplified by the polymerase chain reaction (PCR) in 117 patients

Accepted for publication March 5, 1996.

Address reprint requests to Dr. M. Mayumi, Immunology Division, Jichi Medical School, Minamikawachi-Machi, Tochigi-Ken 329-04, Japan.

with chronic hepatitis C, who were positive for both antibody to HCV (anti-HCV) and HCV RNA in serum, and the prevalence was compared with 67 patients with cirrhosis, of whom 20 had hepatocellular carcinoma, and 50 patients without cirrhosis or carcinoma.

MATERIALS AND METHODS

Patients

One-hundred seventeen patients with HCV-associated chronic liver disease who visited or were admitted to Aikawa Internal Hospital were studied. None of the patients carried hepatitis B surface antigen in the serum or had antibody to human immunodeficiency virus type 1. They included 67 patients with liver cirrhosis (LC) of whom 20 had hepatocellular carcinoma and 50 patients with chronic hepatitis (CH) without LC or hepatocellular carcinoma. They all were positive for anti-HCV and HCV RNA. There were no appreciable differences in the sex ratio between the 67 patients with LC and the 50 patients without LC (male to female: 36:31 vs. 30:20), but the patients with LC were significantly older ($P < 0.001$) than those without LC (62 ± 9 vs. 54 ± 11 years).

The diagnosis of liver disease was confirmed by liver biopsy in accordance with the criteria of de Groote et al. [1968]. All of the 20 patients with hepatocellular carcinoma had LC, and the malignancy was confirmed by elevated levels of alpha-fetoprotein, CT scanning, ultrasonography, and angiography in most of them. For patients who were not diagnosed by these procedures, the pathology was verified on specimens obtained by echo-guided, fine needle biopsy. The study was approved by the Ethics Committee of the hospital and all the patients gave an informed consent.

Markers of HCV and the Other Viral Infections

Anti-HCV was determined by enzyme-linked immunosorbent assay of the second generation (Ortho ELISA II: Ortho Diagnostic Systems, Tokyo, Japan). HCV RNA was tested for by reverse transcription PCR with nested primers deduced from the 5′-noncoding region [Okamoto et al., 1994; Okamoto et al., 1990]. Hepatitis B surface antigen was determined by passive hemagglutination using commercial kits (MyCell, Institute of Immunology Co., Ltd., Tokyo, Japan), and antibody to human immunodeficiency virus type 1 by particle agglutination (SERODIA·HIV, Fuji Rebio, Tokyo, Japan).

HLA Class II Types

Blood (3 ml) was obtained from each patient and mixed with an equal volume of sterile Alsever's solution. Peripheral white blood cells were separated by Ficoll-Paque (Pharmacia-LKB Biotechnology, Uppsala, Sweden), and DNA was extracted from them by partition fractionation with commercial kits (SMITEST DNA extraction kit: Sumitomo Metal Industries, Ltd., Tokyo, Japan). The extracted DNA was dissolved in 30 μl of Tris-HCl buffer (10 mM, pH 8.0) supplemented with 1 mM EDTA to a concentration of 100 ng/μl, and a 2 ~ 10-μl portion was used for typing HLA class II genes.

Typing of DRB1 and DQB1 alleles was carried out with commerical assay kit (SMITEST HLA DNA typing system: Sumitomo Metal Industries, Ltd.) by restriction fragment length polymorphism of HLA class II genes amplified by PCR after the method of Onishi et al. [1993]. The procedure involved the amplification of polymorphic gene regions of DRB1 and DQB1 by PCR with appropriate primers, and the digestion of obtained products with restriction endonucleases. HLA types were estimated by the electrophoretic pattern of fragments which was characteristic of each genotype.

Serving as normal controls were 1216 apparently healthy individuals who were surveyed at the 11th workshop of the Japanese Society for HLA Typing [Akaza et al., 1994]. The survey used the restriction fragment length polymorphism method for the same alleles as studied in the patients.

Statistical Analyses

The frequency between groups was compared using the X^2 test and the Fisher's exact test. The relative risk was estimated by the method of Mantel-Haenzel, and 95% confidence limit by that of Clopper and Pearson [1934].

RESULTS

Differences in HLA DRB1 and DQB1 Types and HLA Class II Haplotypes Between Patients With Chronic Hepatitis C and Controls

Patients with chronic hepatitis C had higher frequency of DRB1*0403 (10% [95% confidence range 6–16%] vs. 4% [3–5%]; $P < 0.01$; relative risk [rr] = 2.7) and DRB1*0405 (39% [31–48%] vs. 25% [25–25%]; $P < 0.001$; rr = 2.0) than in controls (Table I). Likewise, DQB1*0401 (34% [27–43%] vs. 24% [24–24%]; $P < 0.05$; rr = 1.6) was more frequent in patients than in the controls (Table II). When DRB1-DQB1 haplotypes were compared between patients with chronic hepatitis C and controls (Table III), the patients had the DRB1*0405-DQB1*0401 and DRB1*0403-DQB1*0302 haplotypes more frequently (34% [27–43%] vs. 21% [21–21%]; $P < 0.01$; rr = 2.0 and 10% [6–16%] vs. 2% [1–2%]; $P < 0.001$; rr = 7.6, respectively).

Differences in HLA DRB1 and DQB1 Types and HLA Class II Haplotypes Among Patients With Chronic Hepatitis C

The distribution of HLA class II alleles was different between the 67 patients with LC, of whom 20 had hepatocellular carcinoma, and the 50 patients with CH who were without LC or hepatocellular carcinoma (Tables I and II). Significant differences were noted in the frequency of some HLA class II alleles. DRB1*0405 (49% [95% confidence range 38–60%] vs. 26% [16–40%]; $P < 0.05$; relative risk [rr] = 2.8) and DQB1*0401 (43% [33–54%] vs. 22% [13–34%]; $P < 0.05$; rr = 2.1) were more frequent in patients with LC than in those without cirrhosis. By contrast, DRB1*0901 (11% [6–19%] vs. 28% [18–40%]; $P < 0.05$; [rr] = 0.3) and DQB1*0303 (11% [16–19%] vs. 36% [25–49%]; $P < 0.01$; rr = 0.2) were less common in patients with LC than in those without LC.

TABLE I. Frequency of HLA Class II DRB1 Alleles in Patients With Chronic Hepatitis C With or Without Liver Cirrhosis

Alleles	CH[a] (n = 117)	Liver cirrhosis: With (n = 67)	Liver cirrhosis: Without (n = 50)	Normal[b] (n = 1216)	Differences: CH vs. Normal	Differences: LC (+)[c] vs. LC (−)[c]
0101	9 (7.7%)	5 (7.5%)	4 (8.0%)	137 (11.3%)		
0401	2 (1.7%)	1 (1.5%)	1 (2.0%)	15 (1.2%)		
0403	12 (10.3%)	9 (13.4%)	3 (6.0%)	50 (4.1%)	$P < 0.01$	
0405	46 (39.3%)	33 (49.3%)	13 (26.0%)	301 (24.8%)	$P < 0.001$	$P < 0.05$
0407	1 (0.9%)	1 (1.5%)	0	17 (1.4%)		
0410	7 (6.0%)	5 (7.5%)	2 (4.0%)	43 (3.5%)		
0701	1 (0.9%)	1 (1.5%)	0	6 (0.5%)		
0802	7 (6.0%)	4 (6.0%)	3 (6.0%)	100 (8.2%)		
0803	12 (10.3%)	6 (9.0%)	6 (12.0%)	193 (15.9%)		
0901	21 (17.9%)	7 (10.5%)	14 (28.0%)	318 (26.2%)		$P < 0.05$
1101	3 (2.6%)	2 (3.0%)	1 (2.0%)	62 (5.1%)		
1201	4 (3.4%)	0	4 (8.0%)	87 (7.2%)		
1202	4 (3.4%)	3 (4.5%)	1 (2.0%)	42 (3.5%)		
1301	2 (1.7%)	2 (3.0%)	0	14 (1.2%)		
1302	20 (17.1%)	9 (13.4%)	11 (22.0%)	160 (13.2%)		
1401	8 (6.8%)	6 (9.0%)	2 (4.0%)	81 (6.7%)		
1403	1 (0.9%)	1 (1.5%)	0	46 (3.8%)		
1405	8 (6.8%)	3 (4.5%)	5 (10.0%)	53 (4.4%)		
1406	4 (3.4%)	1 (1.5%)	3 (6.0%)	41 (3.4%)		
1501	20 (17.1%)	12 (17.9%)	8 (16.0%)	167 (13.7%)		
1502	28 (23.9%)	16 (23.9%)	12 (24.0%)	234 (19.2%)		
1602	2 (1.7%)	2 (3.0%)	0	25 (2.1%)		

[a]CH, total patients with chronic hepatitis C.
[b]Results of large-scale surveys in Japan [Akaza et al., 1994].
[c]LC (+), hepatitis patients with liver cirrhosis; LC (−), hepatitis patients without liver cirrhosis.

TABLE II. Frequency of HLA Class II DQB1 Alleles in Patients With Chronic Hepatitis C With or Without Liver Cirrhosis

Alleles	CH (n = 117)	Liver cirrhosis: With (n = 67)	Liver cirrhosis: Without (n = 50)	Normal (n = 1216)	Differences: CH vs. Normal	Differences: LC (+) vs. LC (−)
0201	1 (0.9%)	1 (1.5%)	0	9 (0.7%)		
0301	17 (14.5%)	9 (13.4%)	8 (16.0%)	266 (21.9%)		
0302	22 (18.8%)	14 (20.9%)	8 (16.0%)	216 (17.8%)		
0303	25 (21.4%)	7 (10.5%)	18 (36.0%)	335 (27.5%)		$P < 0.01$
0401	40 (34.2%)	29 (43.3%)	11 (22.0%)	296 (24.3%)	$P < 0.05$	$P < 0.05$
0402	12 (10.3%)	10 (14.9%)	2 (4.0%)	95 (7.8%)		
0501	9 (7.7%)	5 (7.5%)	4 (8.0%)	154 (12.7%)		
0502	6 (5.1%)	4 (6.0%)	2 (4.0%)	60 (4.9%)		
0503	10 (8.5%)	7 (10.5%)	3 (6.0%)	97 (8.0%)		
0601	39 (33.3%)	21 (31.0%)	18 (36.0%)	401 (33.0%)		
0602	22 (18.8%)	14 (20.9%)	8 (16.0%)	147 (12.1%)		
0603	2 (1.7%)	2 (3.0%)	0	19 (1.6%)		
0604	21 (17.9%)	10 (14.9%)	11 (22.0%)	162 (13.3%)		

The frequency of DRB1*0405 among 1216 controls was lower than among the patients with LC, and that of DRB1*0901 was in between those of patients with and without LC. DQB1*0401 was detected in controls less often than in the patients with LC, while the frequency of DQB1*0303 was in between those of patients with and without LC.

Table III compares DRB1-DQB1 haplotypes among patients with chronic hepatitis C with or without LC, and controls. In patients with chronic hepatitis C, the DRB1*0405–DQB1*0401 haplotype was found more often in the patients with LC than in those without (43% [33–54%] vs. 22% [13–34%]; $P < 0.05$; rr = 2.7). This was contrasted by the DRB1*0901-DQB1*0303 haplotype which was found less often in patients with LC than in those without (9% [4–17%] vs. 28% [18–40%]; $P < 0.05$; rr = 0.3).

Among the normal Japanese population, the frequency of DRB1*0901-DQB1*0303 haplotype was in between those of patients with LC and without LC, while DRB1*0405-DQB1*0401 haplotype was found as frequently in patients without LC.

DISCUSSION

Certain DRB1 and DQB1 alleles in HLA class II and haplotype, such as DRB1*0405 and DQB1*0401 as well as the DRB1*0405-DQB1*0401 haplotype, were found more frequently in 67 patients with chronic hepatitis C with LC than in 50 patients without LC. They all had ongoing HCV infection with anti-HCV and HCV RNA

TABLE III. Frequency of HLA Class II Haplotypes in Patients With Chronic Hepatitis C With or Without Liver Cirrhosis

Haplotype DRB1–DQB1	CH (n = 117)	Liver cirrhosis		Normal (n = 1216)	Differences	
		With (n = 67)	Without (n = 50)		CH vs. Normal	LC (+) vs. LC (−)
0405–0401	40 (34.2%)	29 (43.3%)	11 (22.0%)	255 (21.0%)	$P < 0.01$	$P < 0.05$
1502–0601	28 (23.9%)	16 (23.9%)	12 (24.0%)	211 (17.4%)		
0901–0303	20 (17.1%)	6 (9.0%)	14 (28.0%)	302 (24.8%)		$P < 0.05$
0101–0501	9 (7.7%)	5 (7.5%)	4 (8.0%)	132 (10.9%)		
0803–0601	12 (10.3%)	6 (9.0%)	6 (12.0%)	153 (12.6%)		
1302–0604	20 (17.1%)	9 (13.4%)	11 (22.0%)	116 (9.5%)		
0802–0302	4 (3.4%)	1 (1.5%)	3 (6.0%)	51 (4.2%)		
1501–0602	18 (15.4%)	10 (14.9%)	8 (16.0%)	114 (9.4%)		
1201–0301	3 (2.6%)	0	3 (6.0%)	48 (3.9%)		
1202–0301	4 (3.4%)	3 (4.5%)	1 (2.0%)	34 (2.8%)		
1101–0301	3 (2.6%)	2 (3.0%)	1 (2.0%)	39 (3.2%)		
0406–0302	2 (1.7%)	1 (1.5%)	1 (2.0%)	27 (2.2%)		
1405–0503	5 (5.1%)	3 (4.5%)	3 (6.0%)	24 (2.0%)		
1401–0503	5 (4.3%)	5 (7.5%)	0	24 (2.0%)		
1403–0301	1 (0.9%)	1 (1.5%)	0	43 (3.5%)		
0403–0302	12 (10.3%)	9 (13.4%)	3 (6.0%)	18 (1.5%)	$P < 0.001$	
0802–0402	2 (1.7%)	2 (3.0%)	0	17 (1.4%)		
0410–0402	7 (6.0%)	5 (7.5%)	2 (4.0%)	12 (1.0%)		

in the serum. Among the 67 patients with LC, 20 (30%) had hepatocellular carcinoma, while none of the 50 patients without LC had hepatocellular carcinoma. By contrast, DRB1*0901 and DQB1*0303, as well as the DRB1*0901-DQB1*0303 haplotype, were detected less frequently in patients with LC than in those without cirrhosis.

These results indicate that there are some HLA class II alleles which may be associated with an accelerated progression of chronic hepatitis C. The mechanism of such association is yet to be determined. A role of HLA class II-restricted, CD4+ T-cell immunity directed to HCV proteins has been postulated in HCV infection by others [Botarelli et al., 1993; Minutello et al., 1993; Schupper et al., 1993]. Hepatitis may persist in some individuals with certain HLA class II antigens by means of their association with some HCV epitopes for an efficient recognition by CD4+ T-cells, which would destroy hepatocytes harboring HCV with surface expression of such epitopes. Alternatively, such a recognition might induce protective antibodies to prevent the spread of HCV infection. The latter possibility offers a potential for vaccines involving HCV epitopes recognized by certain HLA class II types.

Apart from differences between hepatitis C patients with and without liver cirrhosis, there were some differences in HLA class II alleles and haplotypes between the patients with chronic hepatitis C and controls in Japan. Thus, both DRB1*0403 and DRB1*0405, as well as DQB1*0401, were more frequent in patients than in controls. Likewise, both DRB1*0405-DQB1*0401 and DRB1*0403-DQB1*0302 haplotypes were more frequent in patients than in controls.

There is accumulating evidence to indicate that like hepatitis B, HCV-associated liver disease may be induced by immune responses of the host [Jung et al., 1994; Rice and Walker, 1995]. Clinically, this is demonstrated by severe hepatitis in HCV-infected individuals with genetic deficiency of immunoglobulins contrasting with no disease in those with decreased cellular immune responses induced either by heredity or by cytotoxic drugs. In vitro experiments to evaluate the stimulation of CD8+ lymphocytes with oligopeptides mimicking class I-restricted oligopeptide motifs will help in understanding the pathogenesis of hepatitis C [Battegay et al., 1995; Cerny et al., 1995; Kita et al., 1993; Koziel et al., 1993; Koziel et al., 1992; Shirai et al., 1995].

DR5 antigen was found more often in symptom-free carriers than in hepatitis C patients in an Italian population, and the antigen was suggested as protective against the development of hepatitis [Peano et al., 1994]. Similarly, DR13 was found more frequently in Japanese HCV carriers with inactive disease than in those with active hepatitis [Kuzushita et al., 1996]. These observations, however, relate to the differences between the patients infected with HCV with clinical symptoms and those who are symptom-free, which is beyond the scope of the present study.

The full picture of cellular immunity to HCV, either of class I-restricted CD8+ or class II-restricted CD4+ T cells, cannot be portrayed merely by evaluating immune responses of these cells against certain epitopes or proteins of HCV. Rather, the configuration of major histocompatibility complex in symptom-free carriers and comparison with that of patients with different types of HCV-associated liver disease might give better insight into the progression of liver disease as well as the prevention of severe disease. There have been only few studies along this line so far [Peano et al., 1994; Verdon et al., 1994].

ACKNOWLEDGMENT

We thank Dr. Hiroshi Okuda of the Department of Legal Medicine and Human Genetics, Jichi Medical School for his advice in the statistical analyses of our

results, and Dr. Michio Imawari of Hepatitis Division, Jichi Medical School for valuable discussion.

REFERENCES

Akaza T, Imanishi T, Fujiwara K, Tokunaga K, Yashiki S, Fujiyoshi T, Sonoda S, Juji T (1994): HLA alleles and haplotypes of Japanese populations. MHC & IRS 1:S219–226.

Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M, Choo QL, Kuo G (1989): Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. New England Journal of Medicine 327: 1899–1905.

Battegay M, Fikes J, Di Bisceglie A, Wentworth PA, Sette A, Celis E, Ching WM, Grakoui A, Rice CM, Kurokohchi K, Berzofsky JA, Hoofnagle JH, Feinstone SM, Akatsuka T (1995): Patients with chronic hepatitis C have circulating cytotoxic T cells which recognize hepatitis C virus-encoded peptides binding to HLA-A2.1 molecules. Journal of Virology 69:2462–2470.

Botarelli P, Brunetto MR, Minutello MA, Calvo P, Unutmaz D, Weiner AJ, Choo QL, Shuster JR, Kuo G, Bonino F, Houghton M, Abrignani S (1993): T-lymphocyte response to hepatitis C virus in different clinical courses of infection. Gastroenterology 104:580–587.

Cerny A, McHutchison JG, Pasquinelli C, Brown ME, Brothers MA, Grabscheid B, Fowler P, Houghton M, Chisari FV (1995): Cytotoxic T lymphocyte response to hepatitis C virus-derived peptides containing the HLA A2.1 binding motif. Journal of Clinical Investigation 95:521–530.

Clopper CJ, Pearson ES (1934): The use of confidence or fiducial limits illustrated in the case of binomial. Biometrika 26:404–414.

de Groote J, Desmet VJ, Gedigk P, Korb G, Popper H, Poulsen H, Scheuer PJ, Schmid M, Thaler H, Uehlinger E (1968): A classification of chronic hepatitis. Lancet ii:626–628.

Dienstag JL (1983): Non-A, non-B hepatitis. I. Recognition, epidemiology, and clinical features. Gastroenterology 85:439–462.

Jung MC, Diepolder HM, Pape GR (1994): T cell recognition of hepatitis B and C viral antigens. European Journal of Clinical Investigation 24:641–650.

Kita H, Moriyama T, Kaneko T, Harase I, Nomura M, Miura H, Nakamura I, Yazaki Y, Imawari M (1993): HLA B44-restricted cytotoxic T lymphocytes recognizing an epitope on hepatitis C virus nucleocapsid protein. Hepatology 18:1039–1044.

Koziel MJ, Dudley D, Afdhal N, Choo QL, Houghton M, Ralston R, Walker BD (1993): Hepatitis C virus (HCV)-specific cytotoxic T lymphocytes recognize epitopes in the core and envelope proteins of HCV. Journal of Virology 67:7522–7532.

Koziel MJ, Dudley D, Wong JT, Dienstag J, Houghton M, Ralston R, Walker BD (1992); Intrahepatic cytotoxic T lymphocytes specific for hepatitis C virus in persons with chronic hepatitis. Journal of Immunology 149:3339–3344.

Kuzushita N, Hayashi N, Katayama K, Hiramatsu N, Yasumaru M, Murata H, Shimizu Y, Yamazaki T, Fushimi H, Kotoh K, Kasahara A, Fusamoto H, Kamada T (1996): Increased frequency of HLA DR13 in hepatitis C virus carriers with persistently normal ALT levels. Journal of Medical Virology 48:1–7.

Minutello MA, Pileri P, Unutmaz D, Censini S, Kuo G, Houghton M, Brunetto MR, Bonino F, Abrignani S (1993): Compartmentalization of T lymphocytes to the site of disease: intrahepatic CD4+ T cells specific for the protein NS4 of hepatitis C virus in patients with chronic hepatitis C. Journal of Experimental Medicine 178:17–25.

Okamoto H, Mishiro S, Tokita H, Tsuda F, Miyakawa Y, Mayumi M (1994): Superinfection of chimpanzees carrying hepatitis C virus of genotype II/1b with that of genotype III/2a or I/1a. Hepatology 20:1131–1136.

Okamoto H, Okada S, Sugiyama Y, Tanaka T, Sugai Y, Akahane Y, Machida A, Mishiro W, Yoshizawa H, Miyakawa Y, Mayumi M (1990): Detection of hepatitis C virus RNA by a two-stage polymerase chain reaction with two pairs of primers deduced from the 5′-noncoding region. Japanese Journal of Experimental Medicine 60:215–222.

Onishi H, Iida J, Tanaka H, and Inoko H (1993): SMITEST HLA DNA typing system. MHC & IRS 1:S73–95.

Peano G, Menardi G, Ponzetto A, Fenoglio LM (1994): HLA-DR5 antigen. A genetic factor influencing the outcome of hepatitis C virus infection? Archives of Internal Medicine 154:2733–2736.

Rice CM, and Walker CM (1995): Hepatitis C virus-specific T lymphocyte responses. Current Opinion in Immunology 7:532–538.

Schupper H, Hayashi P, Scheffel J, Aceituno S, Paglieroni T, Holland PV, Zeldis JB (1993): Peripheral-blood mononuclear cell responses to recombinant hepatitis C virus antigens in patients with chronic hepatitis C. Hepatology 18:1055–1060.

Shirai M, Arichi T, Nishioka M, Nomura T, Ikeda K, Kawanishi K, Engelhard V. H., Feinstone SM, Berzofsky JA (1995): CTL responses of HLA-A2.1-transgenic mice specific for hepatitis C viral peptides predict epitopes for CTL of humans carrying HLA-A2.1. Journal of Immunology 154:2733–2742.

Van der Poel C, Reesink HW, Schaasberg W, Leentvaar KA, Bakker E, Exel OP, Lelie PN (1990): Infectivity of blood seropositive for hepatitis C virus antibodies. Lancet 335:558–560.

Verdon R, Pol S, Landais P, Mattlinger B, Carnot F, Brechot C, Busson M, Kreis H (1994): Absence of association between HLA antigens and chronicity of viral hepatitis in haemodialyzed patients. Journal of Hepatology 21:388–393.